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Molecular triggers of egg activation at fertilization in mammals

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Abstract

In mammals, the sperm activates the development of the egg by triggering a series of oscillations in the cytosolic-free Ca^{2+} concentration (Ca^{2+}_i). The sperm triggers these cytosolic Ca^{2+}_i oscillations after sperm–egg membrane fusion, as well as after intracytoplasmic sperm injection (ICSI). These Ca^{2+}_i oscillations are triggered by a protein located inside the sperm. The identity of the sperm protein has been debated over many years, but all the repeatable data now suggest that it is phospholipase C ζ (PLC ζ). The main downstream target of Ca^{2+}_i oscillations is calmodulin-dependent protein kinase II (CAMKII (CAMK2A)), which phosphorylates EMI2 and WEE1B to inactivate the M-phase promoting factor protein kinase activity (MPF) and this ultimately triggers meiotic resumption. A later decline in the activity of mitogen-activated protein kinase (MAPK) then leads to the completion of activation which is marked by the formation of pronuclei and entry into interphase of the first cell cycle. The early cytosolic Ca^{2+}_i increases also trigger exocytosis via a mechanism that does not involve CAMKII. We discuss some recent developments in our understanding of these triggers for egg activation within the framework of cytosolic Ca^{2+}_i signaling.

Reproduction (2016) **152** R41–R50

Introduction

Egg activation refers to the early events that occur at fertilization and that start the development of the embryo. Two of the major events of activation are meiotic resumption and cortical granules exocytosis. In mammalian eggs (metaphase II oocytes), the events of activation are triggered by a transient increase in the cytosolic-free Ca^{2+} concentration (Ca^{2+}_i). In mammals, the Ca^{2+}_i signal consists of a series of repetitive increases that last several hours (Fig. 1). There have been reviews on the roles of other molecules in fertilization including those involved in sperm–egg fusion, or in the changes in the cytoskeleton or the meiotic spindle (Jones 2007, Horner & Wolfner 2008, Clift & Schuh 2013, Okabe 2014). There are also reviews on aspects of Ca^{2+}_i homeostasis during oocyte maturation in preparation for fertilization (Machaca 2007, Wakai & Fissore 2013). Here, we focus on the way the sperm triggers the Ca^{2+}_i signals that activate mammalian eggs. In addition, we shall consider aspects of how Ca^{2+}_i triggers the two most studied events of oocyte activation, namely the completion of meiosis and cortical granule exocytosis. These two events are downstream of the Ca^{2+}_i signal and appear to involve independent pathways.

Ca^{2+}_i oscillations at fertilization

Oscillatory increases in Ca^{2+}_i are both necessary and sufficient for egg activation at fertilization (Kline &

Kline 1992, Ozil *et al.* 2005). In the mouse egg, these oscillations start about 1–2 min after sperm fusion (Lawrence *et al.* 1997). The Ca^{2+} release during the first oscillation originates from the sperm–fusion point and the later transients consist of rapid Ca^{2+}_i waves that sweep across the egg (Deguchi *et al.* 2000). These repetitive Ca^{2+}_i transients, or Ca^{2+}_i spikes, often referred as ‘ Ca^{2+}_i oscillations’, can persist for up to 5 or 6 h, and in mouse eggs, they stop around the time of pronuclei formation (Marangos *et al.* 2003) (Fig. 1). The frequency of sperm-induced Ca^{2+}_i oscillations can vary considerably between species, from one transient every 10–20 min in the mouse zygote, to about one Ca^{2+}_i transient every 30–60 min in bovine or human zygotes (Taylor *et al.* 1993, Fissore *et al.* 1995, Deguchi *et al.* 2000). These are low-frequency Ca^{2+}_i oscillations by comparison to somatic cells.

Ca^{2+}_i oscillations during mammalian fertilization are a result of IP_3 -induced Ca^{2+} release. If IP_3 receptors are inhibited, or their expression downregulated before fertilization, no Ca^{2+}_i oscillations are detected (Miyazaki *et al.* 1992). Furthermore, IP_3 Rs become physiologically downregulated during Ca^{2+}_i oscillations at fertilization in direct response to increases in IP_3 (Brind *et al.* 2000). The most likely explanation for how the sperm initiate IP_3 production and Ca^{2+}_i oscillations is that sperm introduces a diffusible cytosolic factor into the egg after fusion (Swann 1990). This idea is supported by the finding that the injection of soluble cytosolic extracts from sperm can cause Ca^{2+}_i oscillations and egg

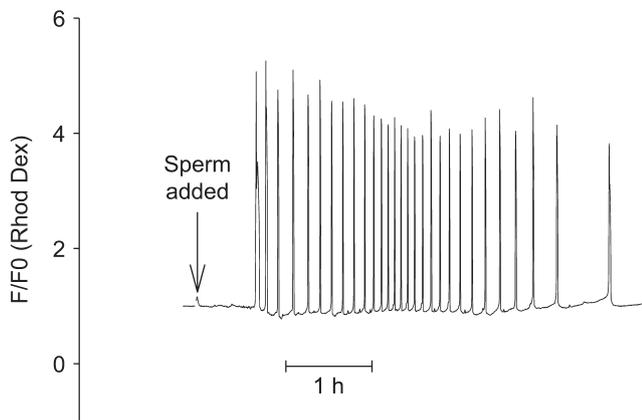


Figure 1 Ca^{2+}_i oscillations at fertilization in a mouse egg. An example is shown of a recording from mouse eggs that were microinjected with the Ca^{2+} -sensitive fluorescent dye Rhod dextran and fluorescence measured every 10s following the addition of capacitated mouse sperm (at the arrow). Further details in (Saunders *et al.* 2002).

activation (Swann 1990). Sperm extracts from different mammalian species are able to activate eggs from different species showing that the factor causing Ca^{2+} release is not species specific (Swann 1994). However, it is sperm specific since extracts from somatic tissues do not trigger Ca^{2+}_i oscillations (Jones *et al.* 2000). The idea of an egg-activating factor inside the sperm is also supported by the clinical practice of ICSI (intracytoplasmic sperm injection), which triggers Ca^{2+}_i oscillations similar to those at fertilization (Tesarik & Sousa 1994). The active factor in these ICSI studies has been referred to as SOAF (sperm-borne oocyte-activating factor) (Kimura *et al.* 1998). The SOAF is associated with the perinuclear matrix, which lies beneath the plasma membrane around the sperm head (Kimura *et al.* 1998). Proteins extracted from the perinuclear matrix also cause Ca^{2+}_i oscillations in eggs (Perry *et al.* 2000). The SOAF is clearly active in different mammals and is active across species and so, for example, injection of human sperm can cause Ca^{2+}_i oscillations in mouse eggs (Yoon *et al.* 2008). These data confirm that mammalian sperm contain a sperm-specific protein(s) that can cause the physiological pattern of Ca^{2+}_i oscillations in eggs seen at fertilization.

Although each increase in cytosolic Ca^{2+} is a result of intracellular Ca^{2+} release, Ca^{2+} influx across the plasma membrane is important in maintaining Ca^{2+}_i oscillations. Therefore, for example, removal of extracellular Ca^{2+} leads to the cessation of Ca^{2+}_i transients in fertilizing hamster and mouse eggs (Igusa & Miyazaki 1983). We also know that Ca^{2+} influx is stimulated during each Ca^{2+}_i increase since Mn^{2+} influx, used as a surrogate for Ca^{2+} , can be detected following each Ca^{2+}_i rise (McGuinness *et al.* 1996). Ca^{2+} influx in mammalian eggs may involve a number of different channels. In mouse eggs, one of these channels is T-type Ca^{2+}

channel CaV 3.2 (Bernhardt *et al.* 2015). This may not be the only channel to bring Ca^{2+} in because CaV 3.2 null female mice are fertile (Bernhardt *et al.* 2015). Another channel in the mouse plasma membrane is the TRPV3 which when stimulated can lead to parthenogenetic egg activation (Carvacho *et al.* 2013, Lee *et al.* 2016). These types of channels are probably the most significant in mouse eggs because store-operated Ca^{2+} influx is downregulated in the mouse oocyte in preparation for fertilization. This is achieved by loss of cortical Stim1 and Orai during oocyte maturation (Lee *et al.* 2013). The reason why the egg has to downregulate the influx seems to be that the release machinery becomes so sensitive to Ca^{2+} -induced Ca^{2+} release that if the influx levels of an immature oocyte are maintained in a mature egg, it causes very high-frequency oscillations that lead to cell death (Lee *et al.* 2013). Although store-operated Ca^{2+} channels does not appear to be the mechanism for Ca^{2+} influx in mouse eggs (Miao *et al.* 2012), it may be significant in some other species. For example, in pig eggs, the experimental downregulation of *STIM1* or *ORAI* leads to premature cessation of Ca^{2+}_i oscillations at fertilization (Wang *et al.* 2015).

Changes in the Ca^{2+} content inside stores have been directly measured using a Ca^{2+} probe targeted to the endoplasmic reticulum in mouse eggs (Takahashi *et al.* 2013, Wakai *et al.* 2013). There is a marked decrease in store Ca^{2+} during each cytosolic Ca^{2+}_i increase, and then gradual refilling of Ca^{2+} stores during the intervals between Ca^{2+}_i spikes (Takahashi *et al.* 2013, Wakai *et al.* 2013). It is suggested that refilling of stores sets the timing of each Ca^{2+}_i increase and this is very likely with relatively high-frequency Ca^{2+}_i oscillations (Takahashi *et al.* 2013, Wakai *et al.* 2013). However, whether Ca^{2+} refilling always acts a pacemaker is unclear. For example, with the initial low-frequency oscillations, the level of Ca^{2+} in the store does not correlate with the time of each Ca^{2+} release event (Wakai *et al.* 2013). Furthermore, several cycles of Ca^{2+} release can occur in the presence of the Ca^{2+} pump inhibitor thapsigargin, which inhibits refilling (Wakai *et al.* 2013). The timing of each Ca^{2+} release event may be determined by a number of factors other than Ca^{2+} store loading.

PLC ζ is the soluble sperm factor and SOAF

The sperm extracts that trigger Ca^{2+}_i oscillations in eggs contain a high phospholipase C (PLC) activity that is distinctive in being stimulated by Ca^{2+} concentrations equivalent to resting levels in eggs (Jones *et al.* 1998, Rice *et al.* 2000). It was then proposed that the sperm factor is some form of PLC (Jones *et al.* 1998, Swann & Parrington 1999). Such a PLC would have to be distinct from the β , γ , or δ isoforms, which are unable, or much less able, to cause Ca^{2+}_i oscillations in eggs and are present in many somatic tissues (Jones *et al.* 1998).

However, mammalian testes do specifically express a distinct PLC isoform known as PLC ζ (zeta) (Saunders *et al.* 2002). Microinjection of PLC ζ , as RNA or protein, causes Ca $^{2+}$ _i oscillations in mouse, cow, pig, or human eggs (Saunders *et al.* 2002, Kouchi *et al.* 2004, Rogers *et al.* 2004, Ross *et al.* 2008, Ito & Kashiwazaki 2012, Nomikos *et al.* 2013b) (Fig. 2). Subsequently, egg activation and development occurs up to the blastocyst stage (Cox *et al.* 2002, Saunders *et al.* 2002, Rogers *et al.* 2004, Yoneda *et al.* 2006). Crucially, PLC ζ is able to cause Ca $^{2+}$ _i oscillations at levels that are comparable to that present in a single sperm (Saunders *et al.* 2002, Ross *et al.* 2008). What is more PLC ζ has been found to localize to the equatorial and post-acrosomal regions of the sperm, where the sperm first makes contact with the egg plasma membrane (Fujimoto *et al.* 2004, Heytens *et al.* 2009, Escoffier *et al.* 2016). Finally, sperm extracts depleted of PLC ζ using an anti-PLC ζ antibody are unable to cause Ca $^{2+}$ _i oscillations when injected into eggs (Saunders *et al.* 2002). These data show that PLC ζ is the previously described soluble sperm factor and that it fits the key criteria for triggering the activation of development.

While the identity of the cytosolic 'soluble' sperm factor has been shown to be PLC ζ , studies on mouse sperm had always suggested that they contain an insoluble factor (SOAF) that was located within the perinuclear theca (Kimura *et al.* 1998). The active SOAF has been extracted from the perinuclear theca using reducing agents and further purification identified PLC ζ as the protein that correlated with the ability to activate mouse eggs (Fujimoto *et al.* 2004). These data clearly suggest that both SOAF and the activity of 'soluble sperm factor' are the same thing: namely

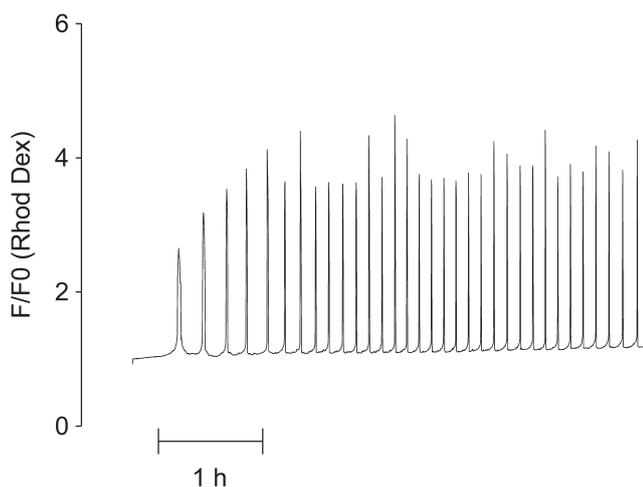


Figure 2 PLC-induced Ca $^{2+}$ _i oscillations in a mouse egg. The conditions are the same as Fig. 1 except that no sperm were added. The egg had been injected with PLC cRNA (0.02 g/L in the pipette) as described in (Nomikos *et al.* 2005). The trace shows a recording over 5 h.

PLC ζ . The explanation for the original differences in their character may be that mouse sperm contain PLC ζ that is tightly bound to the perinuclear matrix, whereas in other species, some of the PLC ζ is more soluble. In boar sperm, for example, it has been shown that PLC ζ is present in both soluble extracts and the sperm perinuclear matrix (Kurokawa *et al.* 2005). It is notable that the analysis of material extracted from the perinuclear matrix provides biochemical confirmation that PLC ζ is localized within the sperm; hence, it is not credible to claim that PLC ζ is a membrane protein (Fujimoto *et al.* 2004, Aarabi *et al.* 2012).

Some of the unique capabilities of PLC ζ in causing Ca $^{2+}$ _i oscillations in eggs can be explained by its structure. PLC ζ consists of X–Y catalytic domains, four EF hand domains, and a C2 domain (Nomikos *et al.* 2005). It also contains an unstructured region between the X and Y domains, referred as the X–Y linker (Nomikos *et al.* 2011). A full review of the relationship between these domains and the activity of PLC ζ in eggs can be found elsewhere (Kouchi *et al.* 2005, Nomikos 2015). Here, we briefly highlight some features of PLC ζ that are pertinent to function in eggs. The EF hand motifs of PLC account for its extraordinary Ca $^{2+}$ sensitivity and allow PLC ζ to generate IP $_3$ at resting levels in the oocyte cytoplasm (Nomikos *et al.* 2015b). Any slight increase in Ca $^{2+}$ _i will also increase IP $_3$ production, and hence, there is a positive-feedback loop of Ca $^{2+}$ _i and IP $_3$ increase. This positive-feedback loop accounts for the enhanced 'Ca $^{2+}$ -induced Ca $^{2+}$ release' after fertilization, and it has been shown to be part of the mechanism generating Ca $^{2+}$ _i oscillations in response to PLC ζ or fertilization (Swann & Yu 2008) (Fig. 3). Furthermore, oscillations in IP $_3$ concentration, in synchrony with Ca $^{2+}$ _i oscillations, have been detected in mouse eggs injected with PLC ζ (Shirakawa *et al.* 2006). The X–Y linker region of PLC ζ is unusual, in that it can account for the ability of PLC ζ to bind to PIP $_2$ (Nomikos *et al.* 2011). For other PLCs, the X–Y linker plays an auto-inhibitory role, but for PLC ζ , the X–Y linker is essential for its binding to its substrate. The role of the C2 domain is currently unknown, but it is important because a chimeric protein made of PLC ζ , but with the C2 domain of PLC δ 1, is unable to cause Ca $^{2+}$ _i oscillations in eggs (Theodoridou *et al.* 2013).

Another unusual feature of PLC ζ is that it appears to be only active in eggs. Expression of PLC ζ in cell lines fails to cause Ca $^{2+}$ _i oscillations and ectopic expression in somatic tissues has surprisingly little effect (Phillips *et al.* 2011). It is possible that the specific effect in eggs is related to an unusual localization pattern. Numerous studies have failed to find PLC ζ in the plasma membrane where most other PLCs localize, and where cells maintain a pool of phosphatidylinositol 4,5-bisphosphate (PIP $_2$), the substrate for PLCs. However, immunocytochemical studies of PLC ζ (at physiological concentrations) have found it localized in multiple vesicles throughout the

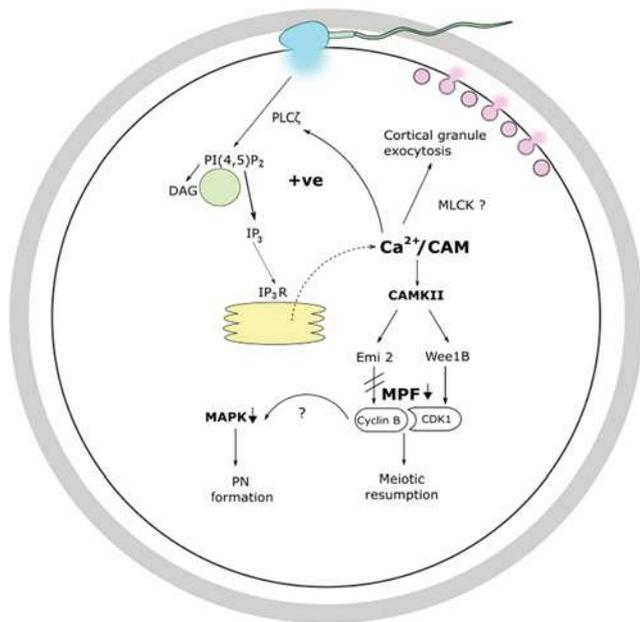


Figure 3 Schematic proposal for the key events triggering egg activation. The sperm fused with the egg and introduces PLC ζ which diffuses into the cytosolic space. Vesicular PIP₂ is hydrolyzed to generate IP₃, which releases Ca²⁺. The Ca²⁺ binds to and activates calmodulin, which stimulates CamKII and possibly other kinases such as MLCK. CamKII phosphorylates EMi2 and WEE1B which ultimately leads to MPF destruction. This in turn causes a much delayed decrease in MAPK activity. PLC activity is further stimulated in response to the increase in Ca²⁺ in the form of a positive-feedback loop (+ve).

egg cytoplasm (Yu *et al.* 2012). There are similar vesicles in mouse eggs that contain PIP₂, and hence, PLC ζ may stimulate PIP₂ hydrolysis from an intracellular source (Yu *et al.* 2012). The implication is that IP₃ and DAG are produced from these internal membranes (Yu *et al.* 2012) (Fig. 3).

So far a genetically modified mouse lacking PLC ζ or containing an inactive PLC ζ mutation has not been reported. One preliminary report suggested that male mice lacking PLC ζ fail to make mature sperm (Ito 2010), but this study has yet to be fully presented. Otherwise, it has been shown that knockdown of the levels of PLC ζ in sperm, using transgenic mice with RNAi, leads to a reduction in Ca²⁺_i oscillations at fertilization suggesting that PLC ζ must play some role in generating oscillations physiologically (Knott *et al.* 2005). The relevance of PLC ζ to fertilization is also suggested from clinical case studies. It was found that there are certain cases of male factor infertility that are associated with reduced levels of PLC ζ (Kashir *et al.* 2011). It has also been reported that one male patient who had repeated failed ICSI had PLC ζ genes with two different mutations on both alleles (Kashir *et al.* 2012b). These mutations were in the catalytic domain of PLC ζ and lead to a loss of its ability to cause Ca²⁺_i oscillations in eggs (Kashir *et al.* 2012b, Escoffier *et al.* 2015). A loss of functionality for PLC ζ on

both alleles is significant since there is a sharing of gene products during spermatogenesis, which means that a mutation on only one PLC ζ allele may not lead to a complete loss of PLC ζ (Kashir *et al.* 2012b). A mutation in the DY19L2 gene, which has previously been associated with the condition globozoospermia, has also been found to correlate with a reduction in the sperm's ability to cause Ca²⁺ oscillations (Escoffier *et al.* 2015). The sperm from patients with this mutation or from Dy19L2-knockout mice is either lacking in PLC ζ or contains much lower levels than would be physiologically expected (Escoffier *et al.* 2015). A recent study by Arnoult and coworkers has emerged of two brothers who also had failed fertilization after ICSI treatments (Escoffier *et al.* 2016). Whole genome sequencing was carried out on these patients. Only one gene was found to have a homologous mutation that was predicted to be disruptive, and that gene was PLC ζ (Escoffier *et al.* 2016). The mutation in this case was in the C2 domain, and it led to a loss of PLC ζ from the sperm as well as reduced ability to cause Ca²⁺_i oscillations (Escoffier *et al.* 2016). This case study provides the strongest evidence to date that PLC ζ is the critical protein for causing Ca²⁺_i oscillations and egg activation at fertilization. This clinical evidence also suggests that PLC ζ sperm levels could be used for a biomarker of fertility and that the levels of PLC ζ within the sperm are vital for successful fertilization and subsequent development. It may be possible that some cases of total fertilization failure that occur following ICSI treatment in the clinic could be a result of reduced levels of PLC ζ in the patient's sperm. The clinical applications for PLC ζ have been discussed thoroughly in a number of reviews (Ramadan *et al.* 2012, Kashir *et al.* 2012a, Nomikos *et al.* 2013a, Swann & Lai 2016).

PAWP is not relevant to egg activation

Another protein called post-acrosomal WW domain-binding protein (PAWP) has been proposed as the factor causing Ca²⁺ release and egg activation at fertilization. PAWP is located in the perinuclear theca of the sperm head, previously identified as containing the SOAF (Kimura *et al.* 1998). It has been reported that recombinant PAWP protein can activate eggs from mice, pigs, frogs, and monkeys (Wu *et al.* 2007, Aarabi *et al.* 2014). Significantly, it was also shown that injection of human recombinant protein PAWP or PAWP cRNA into mouse or human eggs elicited Ca²⁺_i oscillations comparable to those induced by ICSI (Aarabi *et al.* 2014). PAWP protein injection was also reported to induce a Ca²⁺_i increase in frog eggs (Aarabi *et al.* 2010). PAWP is proposed to work via its binding to yes-associated protein (YAP), which then may activate egg-derived PLC ζ by a noncanonical SH3 domain interaction (Wu *et al.* 2007, Aarabi *et al.* 2014). Indeed, injection of a PY-containing peptide into

eggs that competitively binds to YAP abolished ICSI-induced Ca^{2+}_i oscillations and egg activation (Wu *et al.* 2007, Aarabi *et al.* 2014). These data clearly suggest that PAWP could be the physiological sperm factor (or SOAF) causing Ca^{2+}_i oscillations and egg activation.

The above evidence for PAWP at fertilization has, however, now been contradicted by several separate lines of evidence from different research groups. First, as discussed already, the original studies of SOAF identified it as PLC ζ (Fujimoto *et al.* 2004). SOAF activity is present in the perinuclear extracts of the mouse sperm and such extracts were shown to contain several different proteins as well as PLC ζ , but these extracts did not contain PAWP (Fujimoto *et al.* 2004). Secondly, in marked contrast to PLC ζ , the data showing PAWP's ability to cause Ca^{2+}_i increases have not been reproducible (Nomikos *et al.* 2014, 2015a). Independent studies injecting mouse eggs with human or mouse PAWP protein or cRNA failed to detect any Ca^{2+}_i transients (Nomikos *et al.* 2014, 2015a). The expression of PAWP protein from injected RNA was validated in these experiment with tagged or untagged RNA, and the levels that were similar or greater than those levels reported to be found in sperm (Wu *et al.* 2007). In addition, injecting the same PY-containing peptide (used by Aarabi *et al.*) into eggs did not result in any inhibition of Ca^{2+}_i oscillations induced by IVF or ICSI (Nomikos *et al.* 2015a). Thirdly, it has now been shown that PAWP null male mice are fertile and produce sperm that can trigger a normal pattern of Ca^{2+}_i oscillations and embryonic development after ICSI (Satouh *et al.* 2015). Finally, in the above case study of two brothers who have sperm that fail to fertilize eggs in ICSI, and who had a homozygous mutation in PLC ζ , it was found that there were no alterations in the sequence or expression of PAWP (Escoffier *et al.* 2016). These data show that PAWP plays no significant role in generating Ca^{2+}_i oscillations during egg activation in mice or humans.

Downstream of Ca^{2+}_i : meiotic resumption and entry into interphase

The most well-characterized event of mammalian egg activation is the resumption and completion of meiosis which starts with a metaphase-to-anaphase transition, and completes with the formation of two pronuclei (Jones 2007). Meiotic arrest is maintained by high levels of activity of M-phase promoting factor (MPF) that principally consists of cyclin B and a cyclin-dependent kinase (CDK1). The meiotic state also depends on high activity levels of MAPK (Choi *et al.* 1996, Abrieu *et al.* 1997). The key protein linking Ca^{2+}_i oscillations with a decline in MPF activity is calmodulin-dependent protein kinase II (CAMKII (CAMK2A)) (Markoulaki *et al.* 2004). The microinjection of constitutively active CAMKII into mouse eggs triggers meiotic resumption and development up to at least the blastocyst stage

(Knott *et al.* 2006). Moreover, eggs from CAMKII γ knockout mice, or eggs in which CAMKII γ (CAMK2G) has been knocked down, fail to show any signs of meiotic resumption at fertilization (Bacs *et al.* 2010). There are at least two mechanistic pathways linking CAMKII and meiotic resumption. First, active CAMKII phosphorylates EMI2 (Madgwick & Jones 2007), which leads to its phosphorylation by polo kinase, which in turns blocks the ability of EMI2 to inhibit the anaphase-promoting complex (APC). As a consequence, the APC destroys EMI2, as well as cohesion, which holds sister chromatids together, and it destroys cyclin B, which leads to a loss of MPF activity (Hansen *et al.* 2006). The second link between CAMKII and MPF activity involves phosphorylation of the protein kinase WEE1B (Oh *et al.* 2011). WEE1B is a kinase that phosphorylates CDK1 and inhibits MPF activity (Oh *et al.* 2011), and so when WEE1B is phosphorylated, MPF activity falls. In mouse oocytes, WEE1B is essential for inactivation of MPF and cyclin B destruction during oocyte activation (Oh *et al.* 2011). These data suggest a two-pronged action of the Ca^{2+}_i signal on reducing MPF activity (Fig. 3).

While a single Ca^{2+}_i increase at fertilization triggers meiotic resumption, multiple Ca^{2+}_i transients at fertilization are needed to complete the process. A single, physiological sized, Ca^{2+}_i increase can lead to a reduction in MPF activity, but this is only a transient effect (Tatone *et al.* 2002). MPF activity can return after insufficient Ca^{2+}_i increases, and this can lead to a re-establishment of a metaphase arrest: a so-called metaphase III arrest (Kubiak 1989). Using electrical stimulation to mimic the Ca^{2+}_i transients at fertilization, it has been shown that more than eight transients is required to ensure that egg forms pronuclei (Ducibella *et al.* 2002). This is generally consistent with observations on fertilizing mouse eggs, where early termination of Ca^{2+}_i spiking tends to stop 2nd polar body emission and pronuclear formation (Kubiak 1989). It should be noted that Ca^{2+} ionophores only cause a single large Ca^{2+}_i increase in eggs and yet are able to activate development (Winston *et al.* 1991). However, parthenogenetic stimuli that generate a single Ca^{2+}_i transient are not very effective in activating eggs of many species, and particularly poor in activating freshly ovulated eggs (Jones 2007). Ca^{2+} ionophores are generally used in combination with a protein kinase or protein synthesis inhibitor that helps to reduce MPF activity (Jilek *et al.* 2000). Ionophores also cause a much larger and long-lasting Ca^{2+}_i increase than seen physiologically at fertilization.

Many studies of egg activation concern the reinitiation of meiosis. The end of meiosis is marked by the formation of pronuclei and the trigger sequence for this event is less well understood. It is known that the completion of meiosis and entry into interphase depends upon a fall in the activities of MAPK (principally ERK1 and ERK 2) (Moos *et al.* 1996). The activity of ERK1/2 kinase is kept high by phosphorylation by another kinase MEK, which

in turn is kept active through phosphorylation by MOS, which is specifically expressed in oocytes (Dupre *et al.* 2011). A fall in MAPK (ERK1/2) activity is essential for entry into interphase since preventing its decline using phosphatase inhibitors, or by injecting constitutively active MEK, prevents pronuclear formation (Moos *et al.* 1996). By contrast, the MEK inhibitor U0126 induces pronuclear formation (Phillips *et al.* 2002). The trigger for the fall in MAPK activity is the decline in MPF since drugs that inhibit CDK1, such as roscovitine, initiate a decrease in MAPK activity with a delay that mimics fertilization (Gonzalez-Garcia *et al.* 2014). Hence, there is a sequence of triggers in which a decline in MPF leads to a decline in ERK1/2 (MAPK) activity that leads to pronuclear formation (Fig. 3).

The sequence of MPF and MAPK inactivation at fertilization is seen in many vertebrate eggs (Haccard *et al.* 1995, Bogliolo *et al.* 2000, McDougall *et al.* 2011). Nevertheless, the story in mammals is unusual, in that there is a long delay, of several hours, between the fall in MPF activity and the fall in MAPK activity. The use of a luciferase probe of ERK1/2 kinase activity shows that decline in MAPK activity in mouse zygotes is initiated about 2 h after the start of Ca^{2+}_i oscillations, which is about 1.5 h after the decline in cyclin B levels (Gonzalez-Garcia *et al.* 2014). Once started, the decline in MAPK then precedes gradually over the next few hours continuing well after pronuclear formation, which may explain why some reports show a MAPK decline after pronuclear formation (Gonzalez-Garcia *et al.* 2014). Exposing egg to a series of electrical pulses has also shown that MPF can be fully inactivated for about 2 h before a fall in MAP kinase activity is detected (Tatemoto & Muto 2001). Consequently, there is a substantial delay between the fall in activity of MPF and MAPK (Gonzalez-Garcia *et al.* 2014). This delay is not explained by a slow decline in MOS because MOS levels do not decline significantly in the first few hours after fertilization (Gonzalez-Garcia *et al.* 2014). Also, MOS overexpression in mouse eggs does not affect the timing of the fall in ERK1/2 kinase activity (Gonzalez-Garcia *et al.* 2014). The delayed fall in ERK1/2 activity could be driven by an increase in a protein phosphatase activity (Gonzalez-Garcia *et al.* 2014). However, the molecular link between the fall in MPF activity and the stimulation of such phosphatases is unknown.

Downstream of Ca^{2+}_i : cortical granule exocytosis

Another key event of egg activation in mammals is cortical granule exocytosis. The kinetics of exocytosis at fertilization has been accurately measured by membrane capacitance in hamster eggs (Igusa & Miyazaki 1986, Kline & Stewart-Savage 1994). Most of the change in capacitance occurs with the first Ca^{2+}_i increase at fertilization (Kline & Stewart-Savage 1994). Exposing eggs to electrical pulses to generate Ca^{2+}_i increases

also suggests that the first four Ca^{2+}_i spikes trigger the majority of cortical granule to be released. These data are consistent with the role of exocytosis in releasing enzymes that modify the zona pellucida to prevent further sperm entry (Horvath *et al.* 1993). The signals for meiotic resumption are different from those involved in cortical granule exocytosis because injection of constitutively active CamKII triggers meiotic resumption and pronuclear formation, but not exocytosis (Knott *et al.* 2006, Gardner *et al.* 2007, Backs *et al.* 2010). Moreover, fertilization of oocytes from *CAMKII*^{-/-} mice, or from WEE1B knockdown oocytes, does not lead to meiotic resumption, and yet in both cases, cortical granule exocytosis occurs (Ducibella & LeFevre 1997, Backs *et al.* 2010, Oh *et al.* 2011). Hence, meiotic resumption and cortical granule exocytosis appear to be separate downstream events that diverge early on in the Ca^{2+}_i signaling pathway (Fig. 3).

Numerous studies of exocytosis in mammalian eggs have implicated protein kinase C in triggering exocytosis. Increasing PKC activity, for example, using phorbol esters or synthetic DAGs, can stimulate exocytosis in mammalian eggs (Eliyahu & Shalgi 2002). Ca^{2+} ionophores can also stimulate exocytosis in a manner that is blocked by PKC inhibitors (Ducibella & LeFevre 1997). However, the relevance of these results is unclear since PKC inhibitors do not block exocytosis at fertilization in the mouse (Ducibella & LeFevre 1997). Also, both phorbol esters and Ca^{2+} ionophores are nonphysiological in the way they stimulate eggs. For example, a probe made of GFP linked to a DAG-sensing C1 domains shows a distinctive increase in the plasma membrane of mouse eggs in response to phorbol esters, or Ca^{2+} ionophores (Swann & Yu 2008). However, the same probes show no translocation in the plasma membrane at fertilization, or after injection of physiological amounts of PLC ζ (Yu *et al.* 2008). Interestingly, these data suggest that DAG is not produced in significant amounts in the plasma membrane at fertilization in mouse eggs (Halet 2004, Yu *et al.* 2012). This idea is consistent with our suggestion that the PI turnover, and hence DAG production, at fertilization is principally occurring on cytoplasmic vesicles. There may be no DAG produced to stimulate PKC in the plasma membrane at fertilization. The detectable PKC stimulation in the plasma membrane of eggs may be mainly due to a Ca^{2+}_i increase (Halet 2004, Yu *et al.* 2008). One idea is that the PKC and calmodulin pathways converge by the translocation of myristoylated alanine-rich C kinase substrate (MARCKS). Exocytosis is likely to be a multiple stage process that requires the reorganization of the dense actin cytoskeleton in the cortex and the translocation of vesicles to the plasma membrane (Ducibella & Matson 2007). MARCKS has a role in reorganizing actin filaments in the cortex following its translocation either as a result of phosphorylation by PKC or by binding to calmodulin and has been shown to be associated with exocytosis in other cell types as well

as cortical granule exocytosis in eggs (Eliyahu *et al.* 2006, Tsaadon *et al.* 2008). Translocation of the vesicles could involve the Ca^{2+} calmodulin-dependent enzyme myosin light-chain kinase (MLCK). MLCK targets myosin II in neuroendocrine cells and is responsible for translocating vesicles to the synaptic membrane (Ducibella & Matson 2007). Inhibitors of MLCK such as ML7 inhibit cortical granule exocytosis at fertilization in mouse eggs (Matson *et al.* 2006).

Recent studies have shown that cortical granules in mammalian oocytes contain Zn^{2+} (Kim *et al.* 2011, Que *et al.* 2015). At fertilization, or after oocyte activation with Ca^{2+} ionophores, exocytosis triggers release of this Zn^{2+} into the extracellular space, and this release can be detected using fluorescence dyes (Kong *et al.* 2015). It has been proposed that the loss of Zn^{2+} from the egg at fertilization facilitates the process of meiotic resumption (Kim *et al.* 2011). This is plausible since EMI2 is a Zn^{2+} -dependent enzyme and Zn^{2+} chelators, such as TPEN, can trigger meiotic resumption and embryo development (Kim *et al.* 2011). However, for Zn^{2+} released from cortical granules to affect EMI2, there would have to be a decrease in cytosolic Zn^{2+} levels, and it has yet to be shown that cytosolic-free Zn^{2+} levels change in eggs at fertilization. The idea that Zn^{2+} loss via exocytosis plays a role in meiotic resumption is inconsistent with previous studies showing that exocytosis and meiotic resumption are independent events downstream of the Ca^{2+}_i signal.

Conclusions and perspectives

Some of the key molecules in egg activation in mammals are known. CAMKII appears to be cemented in as the hub for all the Ca^{2+} -dependent events triggering meiotic resumption. All the indications are that PLC ζ is the molecule that initiates the Ca^{2+}_i signals that stimulate CAMKII. Questions remain with regards to PLC ζ 's localization and targeting in eggs. We also eagerly await the confirmation phenotype of sperm from a mouse either lacking PLC ζ or else containing an inactive PLC ζ protein. Other important questions that remain concern the nature of Ca^{2+} influx channels and their regulation during the oscillation cycle, as well as the factors determining the long delay between Ca^{2+}_i spikes at fertilization. It will also be interesting to determine the signaling pathway for exocytosis, since it does not appear to involve CAMKII. Finally, we suggest that it is important to establish the link between the fall in MPF and the decline in MAPK activity since it represents the last in a sequence of triggers of egg activation in mammals.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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References

- Aarabi M, Qin Z, Xu W, Mewburn J & Oko R 2010 Sperm-borne protein, PAWP, initiates zygotic development in *Xenopus laevis* by eliciting intracellular calcium release. *Molecular Reproduction and Development* **77** 249–256. (doi:10.1002/mrd.21140)
- Aarabi M, Yu Y, Xu W, Tse MY, Pang SC, Yi YJ, Sutovsky P & Oko R 2012 The testicular and epididymal expression profile of PLCzeta in mouse and human does not support its role as a sperm-borne oocyte activating factor. *PLoS ONE* **7** e33496. (doi:10.1371/journal.pone.0033496)
- Aarabi M, Balakier H, Bashar S, Moskovtsev SI, Sutovsky P, Librach CL & Oko R 2014 Sperm-derived WW domain-binding protein, PAWP, elicits calcium oscillations and oocyte activation in humans and mice. *FASEB Journal* **28** 4434–4440. (doi:10.1096/fj.14-256495)
- Abrieu A, Fisher D, Simon MN, Doree M & Picard A 1997 MAPK inactivation is required for the G2 to M-phase transition of the first mitotic cell cycle. *EMBO Journal* **16** 6407–6413. (doi:10.1093/emboj/16.21.6407)
- Backs J, Stein P, Backs T, Duncan FE, Grueter CE, McAnally J, Qi X, Schultz RM & Olson EN 2010 The gamma isoform of CaM kinase II controls mouse egg activation by regulating cell cycle resumption. *PNAS* **107** 81–86. (doi:10.1073/pnas.0912658106)
- Bernhardt ML, Zhang Y, Erxleben CF, Padilla-Banks E, McDonough CE, Miao YL, Armstrong DL & Williams CJ 2015 CaV3.2 T-type channels mediate Ca^{2+} entry during oocyte maturation and following fertilization. *Journal of Cell Science* **128** 4442–4452. (doi:10.1242/jcs.180026)
- Bogliolo L, Ledda S, Leoni G, Naitana S & Moor RM 2000 Activity of maturation promoting factor (MPF) and mitogen-activated protein kinase (MAPK) after parthenogenetic activation of ovine oocytes. *Cloning* **2** 185–196. (doi:10.1089/152045500454744)
- Brind S, Swann K & Carroll J 2000 Inositol 1,4,5-trisphosphate receptors are downregulated in mouse oocytes in response to sperm or adenophostin A but not to increases in intracellular Ca^{2+} or egg activation. *Developmental Biology* **223** 251–265. (doi:10.1006/dbio.2000.9728)
- Carvacho I, Lee HC, Fissore RA & Clapham DE 2013 TRPV3 channels mediate strontium-induced mouse-egg activation. *Cell Reports* **5** 1375–1386. (doi:10.1016/j.celrep.2013.11.007)
- Choi T, Fukasawa K, Zhou R, Tessarollo L, Borrer K, Resau J & Vande Woude GF 1996 The MOS/mitogen-activated protein kinase (MAPK) pathway regulates the size and degradation of the first polar body in maturing mouse oocytes. *PNAS* **93** 7032–7035. (doi:10.1073/pnas.93.14.7032)
- Clift D & Schuh M 2013 Restarting life: fertilization and the transition from meiosis to mitosis. *Nature Reviews. Molecular Cell Biology* **14** 549–562. (doi:10.1038/nrm3643)
- Cox IJ, Larman MG, Saunders CM, Hashimoto K, Swann K & Lai FA 2002 Sperm phospholipase C ζ from humans and cynomolgus monkeys triggers Ca^{2+} oscillations, activation and development of mouse oocytes. *Reproduction* **124** 611–623. (doi:10.1530/rep.0.1240611)
- Deguchi R, Shirakawa H, Oda S, Mohri T & Miyazaki S 2000 Spatiotemporal analysis of Ca^{2+} waves in relation to the sperm entry site and animal-vegetal axis during Ca^{2+} oscillations in fertilized mouse eggs. *Developmental Biology* **218** 299–313. (doi:10.1006/dbio.1999.9573)
- Ducibella T & LeFevre L 1997 Study of protein kinase C antagonists on cortical granule exocytosis and cell-cycle resumption in fertilized mouse eggs. *Molecular Reproduction and Development* **46** 216–226. (doi:10.1002/(ISSN)1098-2795)
- Ducibella T & Matson S 2007 Secretory mechanisms and Ca^{2+} signaling in gametes: similarities to regulated neuroendocrine secretion in somatic

- cells and involvement in emerging pathologies. *Endocrine Pathology* **18** 191–203. (doi:10.1007/s12022-007-0015-7)
- Ducibella T, Huneau D, Angelichio E, Xu Z, Schultz RM, Kopf GS, Fissore R, Madoux S & Ozil JP** 2002 Egg-to-embryo transition is driven by differential responses to Ca²⁺ oscillation number. *Developmental Biology* **250** 280–291. (doi:10.1006/dbio.2002.0788)
- Dupre A, Haccard O & Jessu C** 2011 MOS in the oocyte: how to use MAPK independently of growth factors and transcription to control meiotic divisions. *Journal of Signal Transduction* **2011** 350412.
- Eliyah E & Shalgi R** 2002 A role for protein kinase C during rat egg activation. *Biology of Reproduction* **67** 189–195. (doi:10.1095/biolreprod67.1.189)
- Eliyah E, Shtraizent N, Tsaadon A & Shalgi R** 2006 Association between myristoylated alanin-rich C kinase substrate (MARCKS) translocation and cortical granule exocytosis in rat eggs. *Reproduction* **131** 221–231. (doi:10.1530/rep.1.00794)
- Escoffier J, Yassine S, Lee HC, Martinez G, Delaroché J, Coutton C, Karaouzene T, Zouari R, Metzler-Guillemain C, Pernet-Gallay K et al.** 2015 Subcellular localization of phospholipase C ζ in human sperm and its absence in DPY19L2-deficient sperm are consistent with its role in oocyte activation. *Molecular Human Reproduction* **21** 157–168. (doi:10.1093/molehr/gau098)
- Escoffier J, Lee HC, Yassine S, Zouari R, Martinez G, Karaouzene T, Coutton C, Kherraf ZE, Halouani L, Triki C et al.** 2016 Homozygous mutation of PLCZ1 leads to defective human oocyte activation and infertility that is not rescued by the WW-binding protein PAWP. *Human Molecular Genetics* **25** 878–891. (doi:10.1093/hmg/ddv617)
- Fissore RA, Pinto-Correia C & Robl JM** 1995 Inositol trisphosphate-induced calcium release in the generation of calcium oscillations in bovine eggs. *Biology of Reproduction* **53** 766–774. (doi:10.1095/biolreprod53.4.766)
- Fujimoto S, Yoshida N, Fukui T, Amanai M, Isobe T, Itagaki C, Izumi T & Perry AC** 2004 Mammalian phospholipase C ζ induces oocyte activation from the sperm perinuclear matrix. *Developmental Biology* **274** 370–383. (doi:10.1016/j.ydbio.2004.07.025)
- Gardner AJ, Knott JG, Jones KT & Evans JP** 2007 CAMKII can participate in but is not sufficient for the establishment of the membrane block to polyspermy in mouse eggs. *Journal of Cellular Physiology* **212** 275–280. (doi:10.1002/jcp.v212:2)
- Gonzalez-Garcia JR, Bradley J, Nomikos M, Paul L, Machaty Z, Lai FA & Swann K** 2014 The dynamics of MAPK inactivation at fertilization in mouse eggs. *Journal of Cell Science* **127** 2749–2760. (doi:10.1242/jcs.145045)
- Haccard O, Lewellyn A, Hartley RS, Erikson E & Maller JL** 1995 Induction of *Xenopus* oocyte meiotic maturation by MAP kinase. *Developmental Biology* **168** 677–682. (doi:10.1006/dbio.1995.1112)
- Halet G** 2004 PKC signaling at fertilization in mammalian eggs. *Biochimica et Biophysica Acta* **1742** 185–189. (doi:10.1016/j.bbamcr.2004.09.012)
- Hansen DV, Tung JJ & Jackson PK** 2006 CAMKII and polo-like kinase 1 sequentially phosphorylate the cytoskeletal factor EMI2/Xerp1 to trigger its destruction and meiotic exit. *PNAS* **103** 608–613. (doi:10.1073/pnas.0509549102)
- Heytens E, Parrington J, Coward K, Young C, Lambrecht S, Yoon SY, Fissore RA, Hamer R, Deane CM, Ruas M et al.** 2009 Reduced amounts and abnormal forms of phospholipase C zeta (PLC ζ) in spermatozoa from infertile men. *Human Reproduction* **24** 2417–2428. (doi:10.1093/humrep/dep207)
- Horner VL & Wolfner MF** 2008 Transitioning from egg to embryo: triggers and mechanisms of egg activation. *Developmental Dynamics* **237** 527–544. (doi:10.1002/(ISSN)1097-0177)
- Horvath PM, Kellom T, Caulfield J & Boldt J** 1993 Mechanistic studies of the plasma membrane block to polyspermy in mouse eggs. *Molecular Reproduction and Development* **34** 65–72. (doi:10.1002/(ISSN)1098-2795)
- Igusa Y & Miyazaki S** 1983 Effects of altered extracellular and intracellular calcium concentration on hyperpolarizing responses of the hamster egg. *Journal of Physiology* **340** 611–632. (doi:10.1113/jphysiol.1983.sp014783)
- Igusa Y & Miyazaki S** 1986 Periodic increase of cytoplasmic free calcium in fertilized hamster eggs measured with calcium-sensitive electrodes. *Journal of Physiology* **377** 193–205. (doi:10.1113/jphysiol.1986.sp016181)
- Ito J & Kashiwazaki N** 2012 Molecular mechanism of fertilization in the pig. *Animal Science Journal* **83** 669–682. (doi:10.1111/asj.2012.83.issue-10)
- Ito M, Nagaoka K & Kuroda K** 2010 Arrest of spermatogenesis at round spermatids in PLC ζ deficient mice. Abstract at the 11th international symposium on spermatology, Okinawa, Japan.
- Jilek F, Huttelova R, Petr J, Holubova M & Rozinek J** 2000 Activation of pig oocytes using calcium ionophore: effect of protein synthesis inhibitor cycloheximide. *Animal Reproduction Science* **63** 101–111. (doi:10.1016/S0378-4320(00)00150-0)
- Jones KT** 2007 Intracellular calcium in the fertilization and development of mammalian eggs. *Clinical and Experimental Pharmacology & Physiology* **34** 1084–1089.
- Jones KT, Cruttwell C, Parrington J & Swann K** 1998 A mammalian sperm cytosolic phospholipase C activity generates inositol trisphosphate and causes Ca²⁺ release in sea urchin egg homogenates. *FEBS Letters* **437** 297–300. (doi:10.1016/S0014-5793(98)01254-X)
- Jones KT, Matsuda M, Parrington J, Katan M & Swann K** 2000 Different Ca²⁺-releasing abilities of sperm extracts compared with tissue extracts and phospholipase C isoforms in sea urchin egg homogenate and mouse eggs. *Biochemical Journal* **346** 743–749. (doi:10.1042/bj3460743)
- Kashir J, Jones C, Lee HC, Rietdorf K, Nikiforaki D, Durrans C, Ruas M, Tee ST, Heindryckx B, Galione A et al.** 2011 Loss of activity mutations in phospholipase C zeta (PLC ζ) abolishes calcium oscillatory ability of human recombinant protein in mouse oocytes. *Human Reproduction* **26** 3372–3387. (doi:10.1093/humrep/der336)
- Kashir J, Jones C & Coward K** 2012a Calcium oscillations, oocyte activation, and phospholipase C zeta. *Advances in Experimental Medicine and Biology* **740** 1095–1121.
- Kashir J, Konstantinidis M, Jones C, Lemmon B, Chang Lee H, Hamer R, Heindryckx B, Deane CM, De Sutter P, Fissore RA et al.** 2012b A maternally inherited autosomal point mutation in human phospholipase C zeta (PLC ζ) leads to male infertility. *Human Reproduction* **27** 222–231.
- Kim AM, Bernhardt ML, Kong BY, Ahn RW, Vogt S, Woodruff TK & O'Halloran TV** 2011 Zinc sparks are triggered by fertilization and facilitate cell cycle resumption in mammalian eggs. *ACS Chemical Biology* **6** 716–723. (doi:10.1021/cb200084y)
- Kimura Y, Yanagimachi R, Kuretake S, Bortkiewicz H, Perry AC & Yanagimachi H** 1998 Analysis of mouse oocyte activation suggests the involvement of sperm perinuclear material. *Biology of Reproduction* **58** 1407–1415. (doi:10.1095/biolreprod58.6.1407)
- Kline D & Kline JT** 1992 Repetitive calcium transients and the role of calcium in exocytosis and cell cycle activation in the mouse egg. *Developmental Biology* **149** 80–89. (doi:10.1016/0012-1606(92)90265-I)
- Kline D & Stewart-Savage J** 1994 The timing of cortical granule fusion, content dispersal, and endocytosis during fertilization of the hamster egg: an electrophysiological and histochemical study. *Developmental Biology* **162** 277–287. (doi:10.1006/dbio.1994.1085)
- Knott JG, Kurokawa M, Fissore RA, Schultz RM & Williams CJ** 2005 Transgenic RNA interference reveals role for mouse sperm phospholipase C ζ in triggering Ca²⁺ oscillations during fertilization. *Biology of Reproduction* **72** 992–996.
- Knott JG, Gardner AJ, Madgwick S, Jones KT, Williams CJ & Schultz RM** 2006 Calmodulin-dependent protein kinase II triggers mouse egg activation and embryo development in the absence of Ca²⁺ oscillations. *Developmental Biology* **296** 388–395. (doi:10.1016/j.ydbio.2006.06.004)
- Kong BY, Duncan FE, Que EL, Xu Y, Vogt S, O'Halloran TV & Woodruff TK** 2015 The inorganic anatomy of the mammalian preimplantation embryo and the requirement of zinc during the first mitotic divisions. *Developmental Dynamics* **244** 935–947. (doi:10.1002/dvdy.v244.8)
- Kouchi Z, Fukami K, Shikano T, Oda S, Nakamura Y, Takenawa T & Miyazaki S** 2004 Recombinant phospholipase C ζ has high Ca²⁺ sensitivity and induces Ca²⁺ oscillations in mouse eggs. *Journal of Biological Chemistry* **279** 10408–10412. (doi:10.1074/jbc.M313801200)
- Kouchi Z, Shikano T, Nakamura Y, Shirakawa H, Fukami K & Miyazaki S** 2005 The role of EF-hand domains and C2 domain in regulation of enzymatic activity of phospholipase C ζ . *Journal of Biological Chemistry* **280** 21015–21021. (doi:10.1074/jbc.M412123200)
- Kubiak JZ** 1989 Mouse oocytes gradually develop the capacity for activation during the metaphase II arrest. *Developmental Biology* **136** 537–545. (doi:10.1016/0012-1606(89)90279-0)

- Kurokawa M, Sato K, Wu H, He C, Malcuit C, Black SJ, Fukami K & Fissore RA 2005 Functional, biochemical, and chromatographic characterization of the complete [Ca²⁺]_i oscillation-inducing activity of porcine sperm. *Developmental Biology* **285** 376–392.
- Lawrence Y, Whitaker M & Swann K 1997 Sperm-egg fusion is the prelude to the initial Ca²⁺ increase at fertilization in the mouse. *Development* **124** 233–241.
- Lee B, Palermo G & Machaca K 2013 Downregulation of store-operated Ca²⁺ entry during mammalian meiosis is required for the egg-to-embryo transition. *Journal of Cell Science* **126** 1672–1681. (doi:10.1242/jcs.121335)
- Lee HC, Yoon SY, Lykke-Hartmann K, Fissore RA & Carvacho I 2016 TRPV3 channels mediate Ca²⁺ influx induced by 2-APB in mouse eggs. *Cell Calcium* **59** 21–31. (doi:10.1016/j.ceca.2015.12.001)
- Machaca K 2007 Ca²⁺ signaling differentiation during oocyte maturation. *Journal of Cellular Physiology* **213** 331–340. (doi:10.1002/(ISSN)1097-4652)
- Madgwick S & Jones KT 2007 How eggs arrest at metaphase II: MPF stabilisation plus APC/C inhibition equals Cytostatic Factor. *Cell Division* **2** 4. (doi:10.1186/1747-1028-2-4)
- Marangos P, FitzHarris G & Carroll J 2003 Ca²⁺ oscillations at fertilization in mammals are regulated by the formation of pronuclei. *Development* **130** 1461–1472. (doi:10.1242/dev.00340)
- Markoulaki S, Matson S & Ducibella T 2004 Fertilization stimulates long-lasting oscillations of CAMKII activity in mouse eggs. *Developmental Biology* **272** 15–25. (doi:10.1016/j.ydbio.2004.04.008)
- Matson S, Markoulaki S & Ducibella T 2006 Antagonists of myosin light chain kinase and of myosin II inhibit specific events of egg activation in fertilized mouse eggs. *Biology of Reproduction* **74** 169–176. (doi:10.1095/biolreprod.105.046409)
- McDougall A, Chenevert J, Lee KW, Hebras C & Dumollard R 2011 Cell cycle in ascidian eggs and embryos. *Results and Problems in Cell Differentiation* **53** 153–169.
- McGuinness OM, Moreton RB, Johnson MH & Berridge MJ 1996 A direct measurement of increased divalent cation influx in fertilised mouse oocytes. *Development* **122** 2199–2206.
- Miao YL, Stein P, Jefferson WN, Padilla-Banks E & Williams CJ 2012 Calcium influx-mediated signaling is required for complete mouse egg activation. *PNAS* **109** 4169–4174. (doi:10.1073/pnas.1112333109)
- Miyazaki S, Yuzaki M, Nakada K, Shirakawa H, Nakanishi S, Nakade S & Mikoshiba K 1992 Block of Ca²⁺ wave and Ca²⁺ oscillation by antibody to the inositol 1,4,5-trisphosphate receptor in fertilized hamster eggs. *Science* **257** 251–255. (doi:10.1126/science.1321497)
- Moos J, Xu Z, Schultz RM & Kopf GS 1996 Regulation of nuclear envelope assembly/disassembly by MAP kinase. *Developmental Biology* **175** 358–361. (doi:10.1006/dbio.1996.0121)
- Nomikos M 2015 Novel signalling mechanism and clinical applications of sperm-specific PLCzeta. *Biochemical Society Transactions* **43** 371–376. (doi:10.1042/BST20140291)
- Nomikos M, Blayney LM, Larman MG, Campbell K, Rossbach A, Saunders CM, Swann K & Lai FA 2005 Role of phospholipase C-zeta domains in Ca²⁺-dependent phosphatidylinositol 4,5-bisphosphate hydrolysis and cytoplasmic Ca²⁺ oscillations. *Journal of Biological Chemistry* **280** 31011–31018. (doi:10.1074/jbc.M500629200)
- Nomikos M, Elgmati K, Theodoridou M, Georgilis A, Gonzalez-Garcia JR, Nounesis G, Swann K & Lai FA 2011 Novel regulation of PLCzeta activity via its XY-linker. *Biochemical Journal* **438** 427–432. (doi:10.1042/BJ20110953)
- Nomikos M, Kashir J, Swann K & Lai FA 2013a Sperm PLCzeta: from structure to Ca²⁺ oscillations, egg activation and therapeutic potential. *FEBS Letters* **587** 3609–3616.
- Nomikos M, Yu Y, Elgmati K, Theodoridou M, Campbell K, Vassilakopoulou V, Zikos C, Livanou E, Amsou N, Nounesis G *et al.* 2013b Phospholipase C-zeta rescues failed oocyte activation in a prototype of male factor infertility. *Fertility and Sterility* **99** 76–85.
- Nomikos M, Sanders JR, Theodoridou M, Kashir J, Matthews E, Nounesis G, Lai FA & Swann K 2014 Sperm-specific post-acrosomal WW-domain binding protein (PAWP) does not cause Ca²⁺ release in mouse oocytes. *Molecular Human Reproduction* **20** 938–947. (doi:10.1093/molehr/gau056)
- Nomikos M, Sanders JR, Kashir J, Sanusi R, Buntwal L, Love D, Ashley P, Sanders D, Knaggs P, Bunkheila A *et al.* 2015a Functional disparity between human PAWP and PLCzeta in the generation of Ca²⁺ oscillations for oocyte activation. *Molecular Human Reproduction* **21** 702–710.
- Nomikos M, Sanders JR, Parthimos D, Buntwal L, Calver BL, Stamatidis P, Smith A, Clue M, Sideratou Z, Swann K *et al.* 2015b Essential role of the EF-hand domain in targeting sperm phospholipase C-zeta to membrane phosphatidylinositol 4,5-bisphosphate (PIP₂). *Journal of Biological Chemistry* **290** 29519–29530.
- Oh JS, Susor A & Conti M 2011 Protein tyrosine kinase WEE1B is essential for metaphase II exit in mouse oocytes. *Science* **332** 462–465. (doi:10.1126/science.1199211)
- Okabe M 2014 Mechanism of fertilization: a modern view. *Experimental Animals* **63** 357–365. (doi:10.1538/expanim.14-0026)
- Ozil JP, Markoulaki S, Toth S, Matson S, Banrezes B, Knott JG, Schultz RM, Huneau D & Ducibella T 2005 Egg activation events are regulated by the duration of a sustained [Ca²⁺]_i signal in the mouse. *Developmental Biology* **282** 39–54. (doi:10.1016/j.ydbio.2005.02.035)
- Perry AC, Wakayama T, Cooke IM & Yanagimachi R 2000 Mammalian oocyte activation by the synergistic action of discrete sperm head components: induction of calcium transients and involvement of proteolysis. *Developmental Biology* **217** 386–393. (doi:10.1006/dbio.1999.9552)
- Phillips KP, Pretunewich MA, Collins JL, Booth RA, Liu XJ & Baltz JM 2002 Inhibition of MEK or cdc2 kinase parthenogenetically activates mouse eggs and yields the same phenotypes as MOS(–/–) parthenogenotes. *Developmental Biology* **247** 210–223. (doi:10.1006/dbio.2002.0680)
- Phillips SV, Yu Y, Rossbach A, Nomikos M, Vassilakopoulou V, Livanou E, Cumbes B, Lai FA, George CH & Swann K 2011 Divergent effect of mammalian PLCzeta in generating Ca²⁺ oscillations in somatic cells compared with eggs. *Biochemical Journal* **438** 545–553. (doi:10.1042/BJ20101581)
- Que EL, Bleher R, Duncan FE, Kong BY, Gleber SC, Vogt S, Chen S, Garwin SA, Bayer AR, Dravid VP *et al.* 2015 Quantitative mapping of zinc fluxes in the mammalian egg reveals the origin of fertilization-induced zinc sparks. *Nature Chemistry* **7** 130–139.
- Ramadan WM, Kashir J, Jones C & Coward K 2012 Oocyte activation and phospholipase C zeta (PLCzeta): diagnostic and therapeutic implications for assisted reproductive technology. *Cell Communication and Signaling* **10** 12. (doi:10.1186/1478-811X-10-12)
- Rice A, Parrington J, Jones KT & Swann K 2000 Mammalian sperm contain a Ca²⁺-sensitive phospholipase C activity that can generate InsP₃ from PIP₂ associated with intracellular organelles. *Developmental Biology* **228** 125–135. (doi:10.1006/dbio.2000.9929)
- Rogers NT, Hobson E, Pickering S, Lai FA, Braude P & Swann K 2004 Phospholipase C-zeta causes Ca²⁺ oscillations and parthenogenetic activation of human oocytes. *Reproduction* **128** 697–702. (doi:10.1530/rep.1.00484)
- Ross PJ, Beyhan Z, Lager AE, Yoon SY, Malcuit C, Schellander K, Fissore RA & Cibelli JB 2008 Parthenogenetic activation of bovine oocytes using bovine and murine phospholipase C zeta. *BMC Developmental Biology* **8** 16. (doi:10.1186/1471-213X-8-16)
- Satouh Y, Nozawa K & Ikawa M 2015 Sperm postacrosomal WW domain-binding protein is not required for mouse egg activation. *Biology of Reproduction* **93** 94. (doi:10.1095/biolreprod.115.131441)
- Saunders CM, Larman MG, Parrington J, Cox LJ, Royle J, Blayney LM, Swann K & Lai FA 2002 PLC ζ : a sperm-specific trigger of Ca²⁺ oscillations in eggs and embryo development. *Development* **129** 3533–3544.
- Shirakawa H, Ito M, Sato M, Umezawa Y & Miyazaki S 2006 Measurement of intracellular IP₃ during Ca²⁺ oscillations in mouse eggs with GFP-based FRET probe. *Biochemical and Biophysical Research Communications* **345** 781–788. (doi:10.1016/j.bbrc.2006.04.133)
- Swann K 1990 A cytosolic sperm factor stimulates repetitive calcium increases and mimics fertilization in hamster eggs. *Development* **110** 1295–1302.
- Swann K 1994 Ca²⁺ oscillations and sensitization of Ca²⁺ release in unfertilized mouse eggs injected with a sperm factor. *Cell Calcium* **15** 331–339. (doi:10.1016/0143-4160(94)90072-8)
- Swann K & Lai FA 2016 The sperm phospholipase C-zeta and Ca²⁺ signalling at fertilization in mammals. *Biochemical Society Transactions* **44** 267–272. (doi:10.1042/BST20150221)

- Swann K & Parrington J** 1999 Mechanism of Ca²⁺ release at fertilization in mammals. *Journal of Experimental Zoology* **285** 267–275. (doi:10.1002/(ISSN)1097-010X)
- Swann K & Yu Y** 2008 The dynamics of calcium oscillations that activate mammalian eggs. *International Journal of Developmental Biology* **52** 585–594. (doi:10.1387/ijdb.072530ks)
- Takahashi T, Kikuchi T, Kidokoro Y & Shirakawa H** 2013 Ca²⁺(+) influx-dependent refilling of intracellular Ca²⁺(+) stores determines the frequency of Ca²⁺(+) oscillations in fertilized mouse eggs. *Biochemical and Biophysical Research Communications* **430** 60–65. (doi:10.1016/j.bbrc.2012.11.024)
- Tatemoto H & Muto N** 2001 Mitogen-activated protein kinase regulates normal transition from metaphase to interphase following parthenogenetic activation in porcine oocytes. *Zygote* **9** 15–23.
- Tatone C, Delle Monache S, Iorio R, Caserta D, Di Cola M & Colonna R** 2002 Possible role for Ca²⁺ calmodulin-dependent protein kinase II as an effector of the fertilization Ca²⁺ signal in mouse oocyte activation. *Molecular Human Reproduction* **8** 750–757. (doi:10.1093/molehr/8.8.750)
- Taylor CT, Lawrence YM, Kingsland CR, Biljan MM & Cuthbertson KS** 1993 Oscillations in intracellular free calcium induced by spermatozoa in human oocytes at fertilization. *Human Reproduction* **8** 2174–2179.
- Tesarik J & Sousa M** 1994 Comparison of Ca²⁺ responses in human oocytes fertilized by subzonal insemination and by intracytoplasmic sperm injection. *Fertility and Sterility* **62** 1197–1204. (doi:10.1016/S0015-0282(16)57185-4)
- Theodoridou M, Nomikos M, Parthimos D, Gonzalez-Garcia JR, Elgmati K, Calver BL, Sideratou Z, Nounesis G, Swann K & Lai FA** 2013 Chimeras of sperm PLCzeta reveal disparate protein domain functions in the generation of intracellular Ca²⁺ oscillations in mammalian eggs at fertilization. *Molecular Human Reproduction* **19** 852–864. (doi:10.1093/molehr/gat070)
- Tsaadon L, Kaplan-Kraicer R & Shalgi R** 2008 Myristoylated alanine-rich C kinase substrate, but not Ca²⁺/calmodulin-dependent protein kinase II, is the mediator in cortical granules exocytosis. *Reproduction* **135** 613–624.
- Wakai T & Fissore RA** 2013 Ca²⁺ homeostasis and regulation of ER Ca²⁺ in mammalian oocytes/eggs. *Cell Calcium* **53** 63–67. (doi:10.1016/j.ceca.2012.11.010)
- Wakai T, Zhang N, Vangheluwe P & Fissore RA** 2013 Regulation of endoplasmic reticulum Ca²⁺ oscillations in mammalian eggs. *Journal of Cell Science* **126** 5714–5724. (doi:10.1242/jcs.136549)
- Wang C, Zhang L, Jaeger LA & Machaty Z** 2015 Store-operated Ca²⁺ entry sustains the fertilization Ca²⁺ signal in pig eggs. *Biology of Reproduction* **93** 25. (doi:10.1095/biolreprod.114.126151)
- Winston N, Johnson M, Pickering S & Braude P** 1991 Parthenogenetic activation and development of fresh and aged human oocytes. *Fertility and Sterility* **56** 904–912. (doi:10.1016/S0015-0282(16)54663-9)
- Wu ATH, Sutovsky P, Manandhar G, Xu W, Katayama M, Day BN, Park K-W, Yi Y-J, Xi YW, Prather RS et al.** 2007 PAWP, a sperm-specific ww domain-binding protein, promotes meiotic resumption and pronuclear development during fertilization. *Journal of Biological Chemistry* **282** 12164–12175. (doi:10.1074/jbc.M609132200)
- Yoneda A, Kashima M, Yoshida S, Terada K, Nakagawa S, Sakamoto A, Hayakawa K, Suzuki K, Ueda J & Watanabe T** 2006 Molecular cloning, testicular postnatal expression, and oocyte-activating potential of porcine phospholipase C ζ . *Reproduction* **132** 393–401. (doi:10.1530/rep.1.01018)
- Yoon SY, Jellerette T, Salicioni AM, Lee HC, Yoo M, Coward K, Parrington J, Grow D, Cibelli JB, Visconti PE et al.** 2008 Human sperm devoid of PLC ζ , zeta 1 fail to induce Ca²⁺ release and are unable to initiate the first step of embryo development. *Journal of Clinical Investigation* **118** 3671–3681.
- Yu Y, Halet G, Lai FA & Swann K** 2008 Regulation of diacylglycerol production and protein kinase C stimulation during sperm- and PLC ζ -mediated mouse egg activation. *Biology of the Cell* **100** 633–643. (doi:10.1042/BC20080033)
- Yu Y, Nomikos M, Theodoridou M, Nounesis G, Lai FA & Swann K** 2012 PLC ζ causes Ca²⁺ oscillations in mouse eggs by targeting intracellular and not plasma membrane PI(4,5)P₂. *Molecular Biology of the Cell* **23** 371–380. (doi:10.1091/mbc.E11-08-0687)

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